Predation-related odours reduce oviposition in a herbivorous mite

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Abstract When adult females of the herbivorous mite, *Tetranychus urticae*, were exposed to the predatory mite, *Phytoseiulus persimilis*, they laid fewer eggs than females that had not been exposed to *P. persimilis* when transferred onto a new leaf patch. However, when *T. urticae* females were exposed to either products of *P. persimilis* or artificially damaged conspecific eggs on a leaf patch, the number of *T. urticae* eggs on a new leaf patch did not differ significantly from the control. The reduced oviposition was neither due to the feeding activity on the leaf patch with *P. persimilis* nor to that on the new leaf patch. There was also no significant difference between the number of *T. urticae* eggs produced on a new leaf patch following exposure to the odours of a neighbouring leaf patch where there had previously been either *P. persimilis* or *T. urticae* adults. However, female *T. urticae* that had been exposed to odours from neighbouring leaf patches on which both *T. urticae* and *P. persimilis* had been placed produced significantly fewer eggs on a new leaf patch than those that had not been exposed to such odours. Neither odours from neighbouring intact leaf patches on which *T. urticae* eggs were preyed on by *P. persimilis*, nor odours from a neighbouring Parafilm patch on which *T. urticae* was preyed on by *P. persimilis* affected the oviposition of *T. urticae*. These data suggest that the presence of *T. urticae*, *P. persimilis* and a leaf patch are needed for the emission of odours to reduce oviposition in *T. urticae*.

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Introduction

Predator-prey interactions do not always result in the consumption of prey by predators (Sih 1980, 1984; Lima and Dill 1990; Lima 1998; Peacor and Werner 2000; Brodin and Johansson 2002; Pangle and Peacor 2006; Werner and Peacor 2006). Prey can escape from predators by reducing their own performance such as growth rate (Lima and Dill 1990; Lima 1998; Peacor and Werner 2000; Brodin and Johansson 2002; Pangle and Peacor 2006; Werner and Peacor 2006) and/or changing their behaviour (Lima 1998; Losey and Denno 1998; Magalhães et al. 2002; Choh and Takabayashi 2007). However, escape from currently invading predators would not be the only goal for predator avoidance for prey over its lifetime, as the prey could encounter predators of the same or different species after the first avoidance. It is widely reported that previous experience with predators affects the subsequent antipredator responses in prey (Chivers et al. 1996; Alvarez and Nicieza 2006; Ferrari et al. 2006; Turner et al. 2006; Dalesman et al. 2006).

Predators attack not only the mobile stages of prey that show avoidance response/behaviour, but also the immobile stages, such as eggs and pupae, which are in most cases more vulnerable to predation than mobile prey. Thus, a reduction in oviposition may be one antipredator behavior for prey animals. It is reported that some females avoid ovipositing near their predators (Faraji et al. 2001; Agarwala et al. 2003; Nomikou et al. 2003), and retain eggs inside of their body in the presence of predators (Montserrat et al. 2007). For example, females of the phytophagous mite *Tetranychus urticae* have been reported to reduce oviposition in the presence of the predatory mite *Phytoseiulus persimilis* (Škaloudová et al. 2007), and to increase migration from a patch with predators to avoid predation (Choh and Takabayashi 2007). However, little is known about whether prior experience of a prey with its predator affects oviposition after escape from the predator. In this study, we examined the oviposition of *T. urticae* in a new patch after experience with *P. persimilis*, and investigated potential cues that could affect the oviposition by *T. uritcae*.

Materials and methods

Plants and mites

Lima bean plants (*Phaseolus lunatus* cv. Pole Sieva) were grown in soil in a greenhouse at $25 \pm 2°C$ and 60–70% relative humidity, under a 16:8 h light:dark photoperiod. For all experiments, plants were 10–15 days post germination. Herbivorous mites (*T. urticae*) were obtained from the Laboratory of Ecological Information, Graduate School of Agriculture, Kyoto University, in 2002 and reared on lima bean plants in a climate-controlled room ($25 \pm 2°C$, 60–70% r.h., 16:8 h L:D). Predatory mites (*P. persimilis*) were purchased from Koppert BV (Berkel en Rodenrijs, The Netherlands). They were reared on detached lima bean leaves that were heavily infested with *T. urticae* under the same climate conditions. Fresh *T. urticae*-infested leaves were added every other day.

General experimental conditions

Leaf patches were prepared for experiments as follows. We cut a piece of leaf from the primary leaf of a lima bean plant with scissors and further cut the piece into 'leaf patches' of three sizes (4 × 5, 2.5 × 4 and 1 × 1 cm). Each piece was placed on water-saturated cotton wool in a Petri dish (9 cm in diameter, 1.4 cm high).

To obtain newly emerged adult female *T. urticae* for oviposition experiments, 50 quiescent female deutonymphs were selected from *T. urticae* cultures and introduced onto a leaf patch (4 × 5 cm). One day after the introduction, 50 adult males were selected from *T. urticae* cultures and introduced onto the leaf patch for mating. For the experiments, we randomly selected newly emerged (2 days post eclosion) *T. urticae* females from the leaf patch. All experiments were performed in a climate-controlled room (25 ± 2°C, 60–70% r.h., 16:8 h L:D).

Effect of the presence of *Phytoseiulus persimilis* on the oviposition of *Tetranychus urticae*

Thirty female *T. urticae* were introduced onto a leaf patch (4 × 5 cm). Ten adult female *P. persimilis* were randomly selected from the culture, and introduced onto the same leaf patch 24 h after the introduction of *T. urticae*. *Phytoseiulus persimilis* individuals were allowed to predate on *T. urticae* and their eggs for 24 h. We observed that a mean (±SE) of 3.08 ± 0.91 *T. urticae* were killed by *P. persimilis* in this period. One day after the introduction of *P. persimilis*, we randomly selected a *T. urticae* individual from the survivors and transferred it onto a new leaf patch (1 × 1 cm). As control, we kept 30 *T. urticae* on a leaf patch (4 × 5 cm) without *P. persimilis* for 2 days, and randomly selected a *T. urticae* female for the new leaf patch. We counted the eggs laid by *T. urticae* on the new leaf patch 3 days after introduction. To exclude the effect of changes in feeding behaviour by *T. urticae* in the presence of *P. persimilis* on the oviposition rate in the new leaf patch, we also exposed 30 female *T. urticae* to 10 *P. persimilis* on a Parafilm patch (4 × 5 cm) for 24 h in the absence of food. As a control, 30 female *T. urticae* were kept on the Parafilm patch in the absence of both *P. persimilis* and food for 24 h. After this, a *T. urticae* female was randomly selected from the Parafilm patch and put on a leaf patch (1 × 1 cm). Each test mite was carefully checked under a stereo microscope to make sure that all body parts were intact. The eggs laid by each test female over 3 days were counted. The experiments were repeated 12 times per treatment. The numbers of eggs were compared with a Mann–Whitney *U*-test.

The leaf area consumed by a female *T. urticae* was examined using individuals that had been exposed to zero and 10 *P. persimilis* on the same leaf patch, as described above. We placed a randomly selected *T. urticae* onto a 1 × 1 cm leaf patch. Each replicate patch was scanned digitally pre and 3 days post introduction of the mite (Kant et al. 2004). Each scan included a 1 cm^2 reference of paper patch. The scans were processed in Photoshop CS2 (Adobe Systems, Mountain View, CA, USA) as follows. First, the background was selected and deleted. Second, the colored pixels were transformed to black-and-white using the threshold tool, so that all damaged areas were set to white and the remaining undamaged leaf-area was set to black. The histogram tool was used to count the white pixels (chlorotic lesions) and the black pixels (undamaged area) of each treated and reference patch. The consumed leaf area was calculated by the difference in the white area pre and 3 days post *T. urticae*-infestation. The experiments were repeated 12 times per treatment. The leaf areas consumed were compared using a Mann–Whitney *U*-test.

Effects of the presence of *Phytoseiulus persimilis* products on the oviposition of *Tetranychus urticae*

Ten *P. persimilis* females were placed on an intact leaf patch (4 × 5 cm). After 24 h, the predatory mites were removed from the patch, but their associated products, such as feces,

were not removed. The patch was called a 'predator-exposed' leaf patch. We then placed 30 adult female *T. urticae* on the predator-exposed leaf patches and kept them for 24 h. As the control, we placed 30 adult female *T. urticae* on an intact leaf patch (4 × 5 cm) and kept them for 24 h. We randomly selected a *T. urticae* female from each of the predator-exposed and control patches, placed them on a separate intact leaf patch (1 × 1 cm) and counted the eggs after 3 days. The experiment was repeated 12 times per treatment. The numbers of eggs were compared with a Mann–Whitney *U*-test.

Effect of the presence of artificially damaged *Tetranychus urticae* eggs on the oviposition of *T. urticae*

Thirty adult female *T. urticae* were placed on a leaf patch (4 × 5 cm) and allowed to lay eggs for 24 h, resulting in more than 300 eggs on the leaf patch. We carefully pierced 50 of these eggs on the leaf patch with a fine needle to imitate *P. persimilis* predation. The female *T. urticae* and damaged eggs were kept on the leaf patch for a further 24 h. As a control, 30 adult female *T. urticae* were kept on a leaf patch of the same size for 48 h, without having damaged any of the eggs. We randomly selected a *T. urticae* female from the patch with damaged eggs and from the control patch, placed them on a separate intact leaf patch (1 × 1 cm) and counted the eggs after 3 days. The experiment was repeated 12 times per treatment. The numbers of eggs were compared with a Mann–Whitney *U*-test.

Effect of predation-related odours on the oviposition of *Tetranychus urticae*

We examined the effects of odours from a leaf patch with either *T. urticae* or *P. persimilis*, and a leaf patch with both *T. urticae* and *P. persimilis*, on the oviposition of *T. urticae*. Two leaf patches (2.5 × 4 cm) were placed 0.5 cm apart in a Petri dish. Thirty adult female *T. urticae* and 10 adult female *P. persimilis* were placed on one of the leaf patches. To prevent the migration of mites to the other leaf, a tanglefoot barrier was made around the edge of the leaf patch. As control, 30 adult female *T. urticae* were placed on one of the leaf patches. Thirty adult female *T. urticae* were introduced onto the other leaf patch without a tanglefoot barrier (hereafter called the exposed patch). Each pair of leaf patches was covered with a plastic cup (12 cm diameter, 6 cm height), which had an air hole (1 × 3 cm, 1.4 cm above the base; see Oku et al. 2003), and kept for 24 h. Under these conditions, *T. urticae* on the exposed leaf patch received odours from the other leaf patch. We randomly selected a *T. urticae* female from the patch that had been placed next to patch with *T. urticae* alone, and *P. persimilis* alone, and the patch with both *T. urticae* and *P. persimilis*, and counted the eggs after 3 days on a separate new intact leaf patch (1 × 1 cm). The experiment was repeated 12 times per treatment. The egg numbers were compared with Steel–Dwass test (Dwass 1960; Steel 1960) following a Kruskal–Wallis test.

We examined the effects of odours from eggs killed by *P. persimilis* on the oviposition of *T. urticae* using the set-up described above. Thirty female *T. urticae* were placed on a leaf patch (2.5 × 4 cm) for 24 h. We then removed all *T. urticae* and placed 10 *P. persimilis* on the leaf patch, and these preyed on the *T. urticae* eggs. This leaf patch was used as the odour source. Thirty *T. uricae* were placed on a separate leaf patch (2.5 × 4 cm), as described above, and received odours from the odour source patch. A leaf patch with unpredated *T. urticae* eggs was used as the odour source for the control patch. After exposure to odours for 24 h, we randomly selected a *T. urticae* from each of the exposed and control patches, placed them on a separate new intact leaf patch (1 × 1 cm) and

counted the eggs after 3 days. The experiment was repeated 12 times per treatment. The egg numbers were compared with a Mann–Whitney U-test.

We also examined the effect of odours from a Parafilm patch (2.5×4 cm) with *T. urticae* and *P. persimilis* on the oviposition of *T. urticae*. Parafilm patches were used to exclude any volatiles of leaf-patch origin. Thirty female *T. urticae* and 10 *P. persimilis* were placed on the Parafilm patch as an odour source. Female *T. urticae* on the exposed leaf patch (2.5×4 cm) received odours from the Parafilm patch for 24 h. As the control, we used a Parafilm patch with *T. urticae* as the odour source. We randomly selected a *T. urticae* from each of the exposed and control patches, placed them on a new intact leaf patch (1×1 cm) and counted the eggs after 3 days. The experiment was repeated 12 times per treatment. The numbers of eggs were compared with a Mann–Whitney U-test.

Results

Effect of the presence of *Phytoseiulus persimilis* on the oviposition of *Tetranychus urticae*

Tetranychus urticae that had been previously exposed to *P. persimilis* on the same leaf patch laid significantly fewer eggs than *T. urticae* that had not been exposed to *P. persimilis* ($U = 26.50$, $df = 1$, $P = 0.0084$; Fig. 1a). When *T. urticae* females were placed on a Parafilm patch without food, they laid fewer eggs when exposed to *P. persimilis* than when unexposed to the predator ($U = 11.50$, $df = 1$, $P = 0.0005$; Fig. 1b). There was no significant difference in the consumed leaf area between *T. urticae* females that had been exposed and unexposed to *P. persimilis* on a leaf patch (unexposed: 0.028 ± 0.0045 cm^2, exposed: 0.035 ± 0.0061 cm^2, $U = 40$, $df = 1$, $P = 0.45$).

Effects of *Phytoseiulus persimilis* products and of artificially damaged *Tetranychus urticae* eggs on the oviposition of *T. urticae*

There was no significant difference in the oviposition rate by *T. urticae* on *P. persimilis*-exposed and clean leaf patches ($U = 60.50$, $df = 1$, $P = 0.51$; Fig. 2a). There was also no
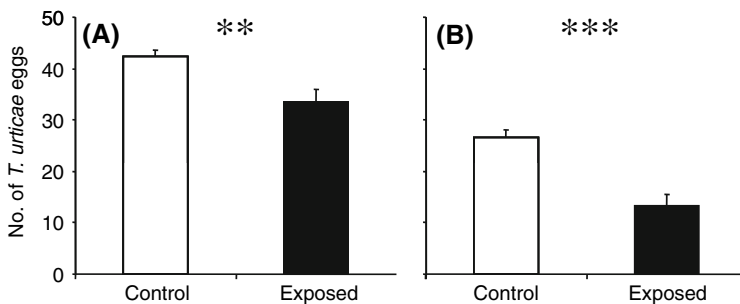

Fig. 1 Mean ($\pm$SE) number of eggs laid by *T. urticae* females on clean leaf patches over 3 days following being kept with and without (control) the predatory mite *P. persimilis* for 1 day on **a** leaf patch and **b** Parafilm patch. The significance of differences were evaluated with a Mann–Whitney U test; **$P < 0.01$, ***$P < 0.001$

Fig. 2 Mean (±SE) number of eggs laid by *T. urticae* female on clean leaf patches over 3 days following being kept for 1 day on a leaf patch **a** exposed to *P. persimilis* and **b** with artificially damaged conspecific eggs

significant difference in oviposition rate by *T. urticae* on patches with and without artificially damaged conspecific eggs ($U = 56.50$, $df = 1$, $P = 0.37$; Fig. 2b).

Effect of predation-related odours on the oviposition of *Tetranychus urticae*

There were significant differences in the oviposition rate of *T. urticae* that had been exposed to odours from adjacent leaf patches of different treatments (*T. urticae*; 45.33 ± 2.01, *P. persimilis*; 44.33 ± 2.04, *T. urticae* + *P. persimilis*; 31.42 ± 3.13, $H_2 = 11.25$, $P < 0.01$, Kruskal–Wallis test). There was no significant difference in oviposition rate by *T. urticae* with previous exposure to odours from a leaf patch with *P. persimilis* compared to that from a leaf patch with conspecifics ($P > 0.05$, Steel–Dwass test). However, there was a significant decrease in oviposition rate by *T. urticae* following exposure to odours from a leaf patch with both *P. persimilis* and *T. urticae* ($P < 0.05$, Steel–Dwass test). There was no difference in oviposition rate by *T. urticae* following exposure to odours from a leaf patch with *T. urticae* eggs and *P. persimilis*, and from a leaf patch with only *T. urticae* eggs ($U = 61.50$, $df = 1$, $P = 0.54$; Fig. 3a). Furthermore, there was no significant difference in oviposition rate between *T. urticae* that had been exposed to odours from a Parafilm patch with *T. urticae* and *P. persimilis*, and from a Parafilm patch with only *T. urticae* ($U = 71.00$, $df = 1$, $P = 0.95$; Fig. 3b).

Fig. 3 Mean (±SE) number of eggs laid by *T. urticae* females over 3 days when kept on a leaf patches next to a **a** leaf patch with *T. urticae* eggs and *P. persimilis* and **b** Parafilm patch with *T. urticae* and *P. persimilis*

Discussion

In this study, *T. urticae* that had previously been exposed to *P. persimilis* showed reduced oviposition on a predator-free leaf patch (Fig. 1a). It has been reported that, in the presence of *P. persimilis*, *T. urticae* reduced its feeding time (Janssen et al. 1997) and increased its moving time (Škaloudová et al. 2007). Such changes in feeding by *T. urticae* might affect oviposition rate on clean leaf patches. In the present study, however, *T. urticae* that had been exposed to *P. persimilis* in the absence of food also reduced oviposition on clean leaf patches (Fig. 1b). These results suggest that feeding changes by *T. urticae* during exposure to *P. persimilis* do not affect oviposition on a new leaf. It is reported that the oviposition of *T. urticae* is related to feeding (Agrawal et al. 2002). If *T. urticae* reduced feeding on clean leaf patches after exposure to *P. persimilis*, they might reduce oviposition. However, our data indicate that reduced oviposition does not result from reduced feeding, because there was no difference in the consumed leaf area between *T. urticae* that had been exposed and not exposed to *P. persimilis*. We already reported that *T. urticae* disperse from a currently inhabiting leaf patch with *P. persimilis* to a new intact leaf patch (Choh and Takabayashi 2007). In this study, we showed that *T. urticae* reduce oviposition on the new patch. Here, some individuals may leave the current patch earlier than the others, and if so, an effect of the duration of the exposure to *P. persimilis* on the oviposition of *T. urticae* in a new patch might be expected. Further studies are needed to explore this possibility.

We examined which cues resulted in the reduced oviposition of *T. urticae*. Škaloudová et al. (2007) reported that *T. urticae* reduced oviposition on a *P. persimilis*-exposed leaf patch. Furthermore, it has been reported that *T. urticae* avoids leaf patches previously exposed to *P. persimilis* or with injured conspecifics (Kriesch and Dicke 1997; Grostal and Dicke 1999). However, we found that *T. urticae* did not reduce oviposition on a clean leaf patch after being kept on a *P. persimilis*-exposed leaf patch or on a leaf patch with damaged conspecific eggs (Fig. 2). In the damaged egg experiment, eggs were pierced in a short time (within 10 min), which is different from the time taken for egg predation. Such a difference might be a factor affecting the reduced oviposition. We then examined whether reduced oviposition was induced by airborne cues such as predator- and predation-related odours. Although *T. urticae* did not reduce oviposition after exposure to odours from a leaf patch with either predators or conspecific eggs killed by *P. persimilis*, oviposition was reduced after exposure to odours from a leaf patch with both predators and *T. urticae*. Furthermore, odours from leaf patches with *T. urticae* and from Parafilm patches with *T. urticae* and *P. persimilis* did not reduce the oviposition of *T. urticae*. These results suggest that odours from leaf patches, *T. urticae* and *P. persimilis* can reduce oviposition. It is important, therefore, to identify the origin and chemical nature of the active component(s) affecting oviposition by *T. urticae*.

Tetranychus urticae eggs are more vulnerable to predation by *P. persimilis* compared to the adult and larvae/nymph stages (Blackwood et al. 2001). It has been reported that female arthropods avoid ovipositing near their predators to reduce the risk of egg predation (Faraji et al. 2001; Agarwala et al. 2003; Nomikou et al. 2003). A *T. urticae* individual that has had experience with predators might reduce oviposition in a predator-free patch to reduce the potential predation risk of their eggs. Further studies are needed to clarify whether the reduced oviposition of *T. urticae* is a strategy to protect offspring.

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